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# DETAILED COMPARATIVE MICROMORPHOLOGICAL AND MICROMETRIC EVALUATION OF *ANDROGRAPHIS PANICULATA* AND ITS ADULTARANT (*A. echioides*)

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## ABSTRACT

*Kalmegha*, the king of bitters belongs to family Acanthaceae. *Kalmegha* is well known medicinal plant which used as potent hepatoprotective drug. In present scenario, due to increase in demand and shortage of supply kalmegh drug, many adulterants are used. One such adulterant is *Andrographis echioides*. Research work has proved that Adulterants of *Kalmegha Andrographis paniculata* is *Andrographis echioides* Nees. where in andrographolide is not present. To overcome from adulterants, present study has been taken with an aim to find satisfactory difference for aiding identification of plant material even in powder form. Material was collected in fresh forms and methods were followed as per standard protocol. The pollen grains present in *A. pannniculata* powder was dumb-bell shaped with two protuberances where as in *A.echioides* it was round with thick wall. Oval shaped cystoliths present in root of *A. pannniculata* measures about 1.2X0.8 µm which was absent in case of *A. echioides.* The micrometrical evaluation along with some differential characters observed in powder microscopy can be helpful in identifying adulteration of *A. paniculata.*

**KEYWORDS:** *Andrographis*, micromorphology, micrometric, pollen grain, *A. echioides*

## INTRODUCTION

*Andrographis panniculata* Nees. is very well knows as “King of Bitters”. It belongs to family Acanthaceae.[1] In Ayurveda, it is used as best and potent hepatoprotective drug, its Sanskrit

name is *Kalmegha*.[2] Due to shortage of drug and increase in the demand of *Kalmegha*, many adulterants are used. *Kalmegha* is adultered with *Andrographis echiodes*, found in tropical India and in dry districts of Maharashtra, Rajasthan and Tamil Nadu.[3] Adulterants of (*Kalmegha*) *Andrographis panniculata* is *Andrographis echioides* Nees., where in andrographolide is not present.[4] *Kalmegha* powder has diacytic stomata, trichomes both glandular and covering trichomes fibers.[5] In present study, *A. panniculata* is compared with *A. echioides*, with an aim to find satisfactory difference for aiding identification of plant material even in powder form.

## MATERIALS AND METHOD

### Collection

The fresh flowering twigs of *Andrographis paniculata* (Burm.f.) Wall. ex Nees. (*Kalmegha*) and *Andrographis echioides* Nees. were collected from Botanical garden of Gujarat Ayurved University located near Sasoi dam, in Jamnagar.

### Macroscopic evaluation.[6, 7]

The morphology of flowering and fruiting twigs of both the species under study was verified with existing flora for authentication.

### Microscopic evaluation.[8, 9]

Free hand transverse sections of root stem and leaves of both the plants were taken, cleared with chloral hydrate, observed under distilled water. Microphotographs were taken by using Carl Zeiss trinocular microscope attached with camera.

### Surface Study.[8, 9]

Leaf surfaces are studied by scraping and also peeling up of both the surfaces of leaves, then washed with chloral hydrate and observed under microscope with distilled water for type of stomata and type of cystoliths.

### Micrometry.[10]

Systematic evaluation and scientific micrometric readings of characters such as diameter of transverse sections including cellular parts were studied. Microphotographs were taken under Carl Zeiss trinocular microscope attached with camera with preloaded micrometric analysis software; All determinations were performed in triplicate and the results are presented as mean value.

## RESULTS AND DISCUSSIONS

### Morphology

The micro morphological measurements of the both plants *Andrographis panniculata* **(**Burm,f.) Nees and *Andrographis echioides* (L.) Nees. are depicted in table no. 1. [Figure 1]

**Table: 1 Shows micro-morphological difference between the plants**

| Morphological characters | *A. panniculata* | *A. echioides* |
|---|---|---|
| **Root:** | Tap root, forming secondary and tertiary branching. | Tap root, not much branched. |
| **Stem:** | Quadrangular, smooth and without hairs | Quadrangular, densely covered with hairs. |
| **Intermodal length:** | mean value 2.46 cm | mean value 1.8 cm |
| **Leaf size:** | 4.6 X1.6 cm | Varies 3.5-3.7 X 1.2-1.4 cm |
| **Leaf shape:** | lanceolate | oblong to elliptic |
| **Leaf base & apex:** | acute at both ends | sub obtuse |
| **Leaf texture:** | glaborous | densely covered with hair |
| **Leaf margin:** | entire | entire |
| **Flower:** | pubescent panicles | pubescent panicles |
| **Pedicel:** | 2-2.5 cm long | 0.8-1.4 cm long |
| **Calyx:** | sepals 5, calyx lobes measure 0.4 cm | sepals 5, calyx lobes measure 1.1cm, densely hirsuit, very slender, axillary unilateral |
| **Corolla:** | petals bilipped, | petals bilipped, |
| **Fruit:** | Capsule with jaculator | Capsule with jaculator |
| **Fruit size:** | 1.6X0.4cm, non hairy. | 1.2 X 0.3cm, hairy. |
| **Seeds:** | Small, brownish after drying, | large, dark, deeply pitted, retinacula spine like |


**Figure 1**

1.1 *A. paniculata* mature flowering and fruiting twig.

1.2 *A. echioides* mature flowering and fruiting twig.

1.3 Comparative of *A. paniculata* and *A. echioides*.

1.4 Comparative photograph of fruit in closer view.

1.5 Opened ripe fruit of *A. paniculata* (dissecting microscope).

1.6 Opened ripe fruit of *A. echioides* (dissecting microscope)

**Microscopy**

***Andrographis panniculata***

**T.S. of root shows**

Single layered barrel shaped epiblema cells often enlarged due to presence of cystoliths of calcium carbonate followed by 5-6 layers of cortical parenchyma cells randomly filled with cystoliths and oil globules. Endodermis single layered, followed by centrally located xylem along with xylem parenchyma, medullary rays and phloem. The central xylem parenchymatous cells showed pitting. [Figure 2 2.1-2.3]

**T.S. of stem shows**

Predominant quadrangular stem consisting of single layered square shaped epidermis covered with thin layer of cuticle often intercepted by sessile glandular trichome and cystoliths. 3-4 layer of hypodermis made up of chlorenchymatous cells, followed by 5-6 layers of cortical cells, at all the four angles beneath the epidermis several layers of parenchyma apart from the cortex is found, single layered endodermis and unevenly distributed pericyclic fibres below which circular vascular bundle is located. Vascular bundle consists of xylem, xylem parenchyma and phloem. Centrally located pith covers up to ¼ $^{th}$ of the section, and often filled with microcrystals and oil globules. [Figure 2 2.4-2.6].

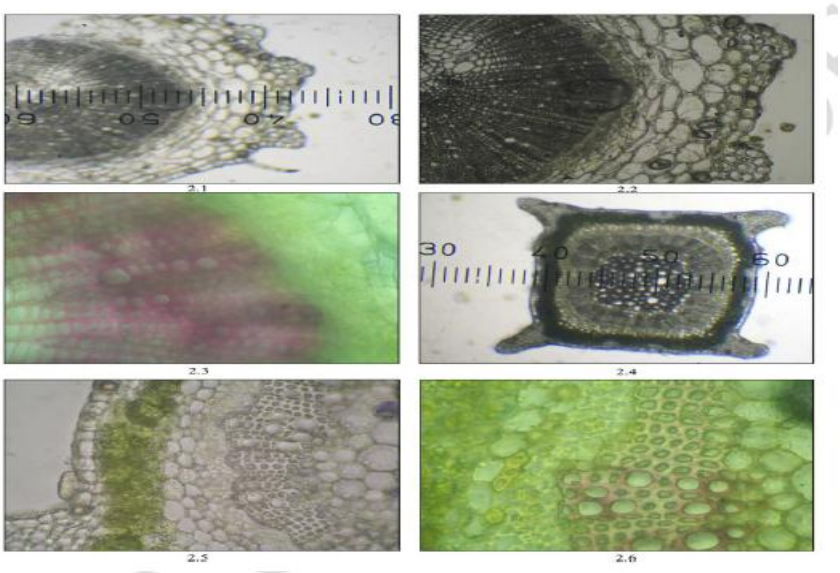

**Figure 2**

2.1 T.S. of root of *A. paniculata* (micromeasurement)

2.2 T.S. of root of *A. paniculata* (10 X)

2.3 shows central xylem of *A. paniculata*

2.4 T.S. of stem of *A. paniculata* (micromeasurement)

2.5 T.S. of stem of *A. paniculata* (10 X)

2.6 shows pericyclic fibre, phloem and xylem.

**T.S. of leaf passing through mid-rib shows**

The diagrammatic T.S. shows laterally extended lamina region through mid rib shows centrally located vascular bundle.

Single layer upper epidermal cells are interrupted by few cystoliths and sessile glandular head trichomes, lower epidermis single layered cells smaller than upper epidermis. Lamina consists of upper single layered barrel shaped palisade parenchyma cells filled with oil globules and cholorphyll content. Lower 5-6 layers of spongy parenchyma cells filled with cholorphyll. Lower epidermis interrupted by stomata, both the epidermis covered by thin cuticle.

T.S. through mid rib shows upper epidermis followed by3-4 layer of collenchymatous cells, gives mechanical support to the centrally situated vascular bundle which consist of xylem and phloem covered by ground tissue from both sides, lower epidermal cells smaller and often intercepted by cystoliths. [Figure 3 3.1-3.4]

**Surface study:** lower epidermis shows diacyctic stomata in abundance interrupted by enlongated cystoliths having blunt end as well as oval shaped cystoliths. Upper epidermis shows parenchyma cells with chlorophyll contents, and intercepted by oval to enlogated cystoliths, glandular sessile trichomes and warty multicellular trichomes, prismatic crystals and oil globules. diacytic stomata were found to be absent. Measurement of trichomes and cystolith are given in table no2. [Figure 3 3.5-3.6].

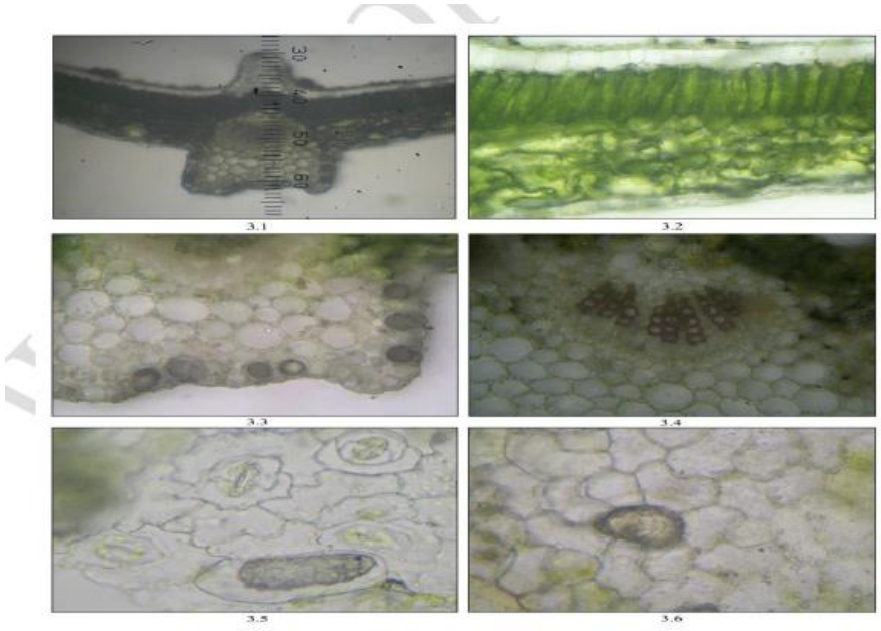

**Figure 3**

3.1 T.S. of leaf of *A. paniculata* (micromeasurement)

3.2 T.S. of lamina of *A. paniculata*

3.3 shows cystoliths at lower epidermis (midrib)

3.4 shows central stellar region (mid rib)

3.5 shows stomata in lower epidermis (surface view)

3.6 shows cystolith in upper epidermis (surface view)

***Andrographis echioides* (L.) Nees.**

**T.S. of root shows**

Single layered uneven shaped epiblema cells devoid of cystoliths, followed by 6-7 layers of cortical parenchyma cells filled with few oil globules. Endodermis single layered, followed by centrally located xylem along with xylem parenchyma, medullary rays and phloem. [Figure 4 4.1-4.3].

**T.S. of stem shows**

T.S. of Stem swollen and slight quadrangular at edges consisting of single layered barrel shaped epidermis covered with very thin layer of cuticle often intercepted by non glandular multicelluar trichome and cystoliths. 2-3layer of hypodermis made up of cholorenchymatous cells, followed by 1-2 layers of cortical cells, single layered endodermis and isolated 1-2 celled pericyclic fibres distributed all around the endodermis below which circular vascular bundle is located. Vascular bundle consists of xylem, xylem parenchyma and phloem.

Centrally located pith is made up of parenchyma cells devoid of any cellular contents. [Figure 4 4.4-4.6].

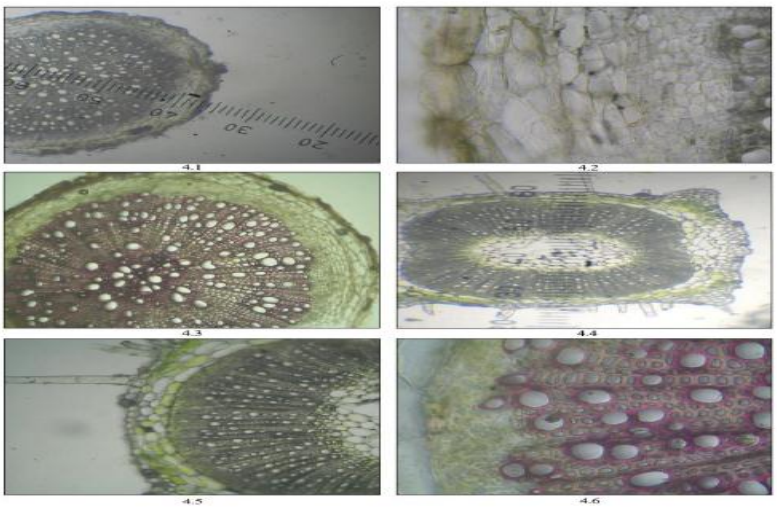

**Figure 4**

4.1 T.S. of root of *A.* echioides (micromeasurement)

4.2 T.S. of root of *A. echioides* (10X)

4.3 showing central xylem region liginified

4.4 T.S. of stem of *A. echioides* (micromeasurement)

4.5 T.S. of stem of *A. echioides* (10X)

4.6 shows phloem, xylem & xylem parenchyma

**T.S. of leaf shows**

The diagrammatic T.S. shows 'w' shaped winged lamina region through mid rib shows centrally located vascular bundle.

Single layer upper epidermal cells interrupted by few cystoliths, epidermis covered with thin cuticle rarely interrupted by stomata. Lamina consists of upper single layer of palisade cells without any intra cellular spaces, with oil globules and chlorophyll pigments. 5-6 layers of spongy parenchyma cells at lower region filled with chlorophyll with large intra cellular spaces some of the cells lead to stomatal openings.

Trough mid rib, below the epidermis followed by 3-4 layers of collenchymatous cells, gives mechanical support to the centrally situated vascular bundle which consists of xylem and phloem covered by ground tissue from both sides, lower epidermal cells smaller and often intercepted by cystoliths. [Fig 5 5.1-5.4].

**Surface study**

Lower epidermis shows diacyctic stomata, enlongated cystoliths, parenchymatous cells filled with oil globules and candelabra trichomes. Upper epidermis shows parenchyma cells with cholorphyll contents, rarely diacytic stomata are observed, cigar shaped cystoliths, pleuricellular trichomes, and oil globules. Measurement of trichomes and crystals are given in table no.2 [Figure 5 5.5-5.6].

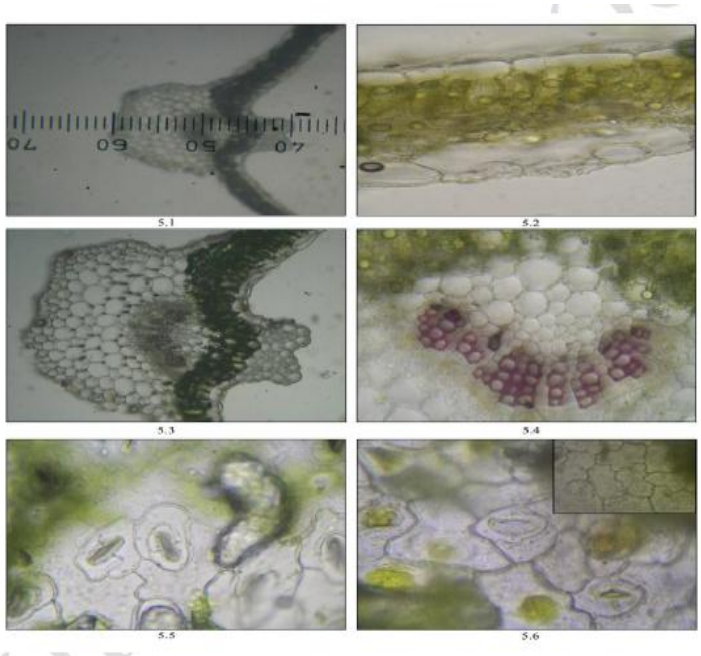

**Figure 5**

5.1 T.S. of leaf of *A.* echioides (micromeasurement)

5.2 T.S. of lamina of *A. echioides*

5.3 T.S. of midrib (10X)

5.4 shows central xylem region.

5.5 shows stomata in lower epidermal surface

5.6 shows stomata and oil globules upper epidermal surface

**Table : 2 comparative surface study of leaf along with micrometric values**

| **Characters** | | ***A. panniculata*** | | ***A. echioides*** | |
|---|---|---|---|---|---|
| | | **Upper surface** | **Lower surface** | **Upper surface** | **Lower surface** |
| **Leaf** | **stomata** | nil | 0.8 X0.8 | 0.9X0.6 µm | 0.7 X1 µm |
| | **Trichome** | 2.75X0.85 µm (warty) 1µm (glandular sessile head) | nil | nil | 4.8X0.2 µm (candelabra trichome) |
| | **cystolith** | 1.5X0.7, 0.9X0.9 µm (round ) | 1.X0.5, 0.8 X 0.6 µm (oval shaped) | 3.1 X1.1 µm | 2.7X0.7µm |

**Powder microscopy**

***Andrographis panniculata***

**Organoleptic characters**

Colour, odour, taste and touch are depicted in table no.3

**Table : 3 shows comparative organoleptic characters**

| **Organoleptic characters** | ***A. panniculata*** | ***A.echioides*** |
|---|---|---|
| **Colour** | Dark green | green |
| **Odour** | Characteristic bitter fumes | characteristic |
| **Taste** | bitter | astringent |
| **Touch** | Rough , fibrous | Rough, coarse |

Diagnostic whole plant powder characters are as follows Spiral vessel, covering trichome, blunt cystoliths, fragment of diacyctic stomata, cork cells from root in tangential view, cork in surface view, sclereid, prismatic crystal, round cystolith, parenchyma cells with oil g., wavy parenchyma of petal, lignified fibres, warty multicellular trichome, fragment of annular and scalriform vessel of stem, sessile glandular trichome in surface view from stem, pollen grain and candalebra trichomes. [Figure6].

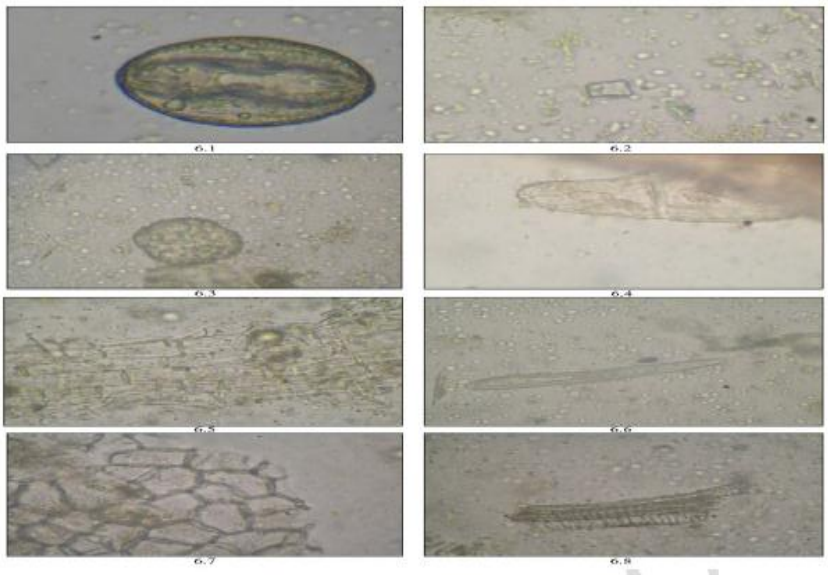

**Figure 6**

6.1 pollen grain *A. paniculata*

6.2 micro prismatic crystals *A. panniculata*

6.3 Round cystolith *A. panniculata*

6.4 multicellular warty trichome *A. panniculata*

6.5 epidermal cells in tangential view

6.6 scleried

6.7 epidermal cells in surface view

6.8 fragment of annular vessel.

***Andrographis echioides* (L.) Nees.**

**Organoleptic characters**

Colour, odour, taste and touch are depicted in table no.3

Diagnostic whole plant powder characters are as follows. Fragment of diacyctic stomata, cystolith- sharp at one end and blunt at the other, fragment of candalebra trichome, palisade cells of flower, group of simple starch grains, brown content, non glandular multicellular trichome, epidermal cells of leaf in surface view, fragment of pitted vessel of root, pollen grain, oil globule, fragment of covering Trichome, multicellular sessile glandular trichomes.[Figure 7].

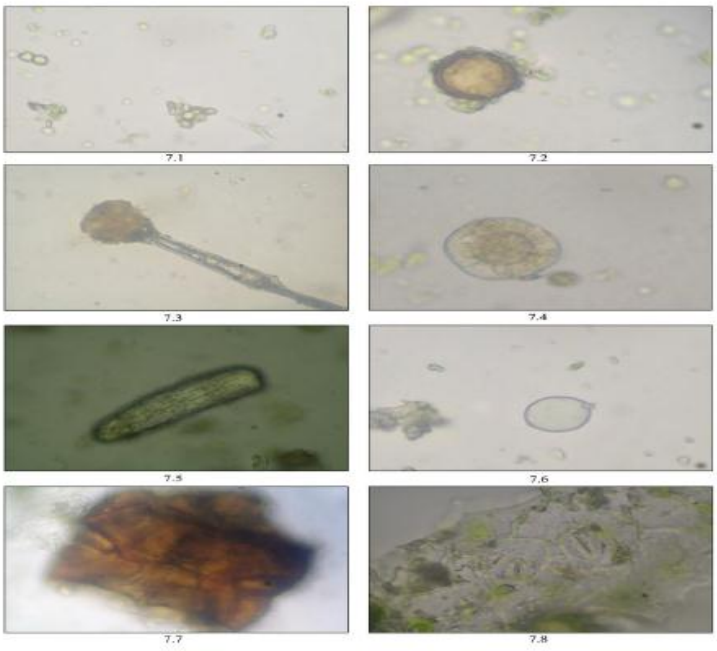

**Figure 7**

7.1 starch grains in group *A. echioides*

7.2 pollen grain

7.3 candelabra trichome fragment

7.4 sessile glandular trichome (surface view)

7.5 elongated cystolith

7.6 oil globule

7.7 dark brown content

7.8 stomata in surface view

**Micrometry**

Micrometery is one of the differentiating tools useful for distinguishing species, The characters like T.S. etc. are mentioned in table no.4. [Figure 8].

**Table : 4 shows comparative micrometric values of various microscopic characters**

| **Characters** | ***A. panniculata*** | ***A.echioides*** |
|---|---|---|
| **T.S. of Root** | 2.1µm$^2$ | 2 µm$^2$(4xX10x) |
| **T.S. of Stem** | 1.2 µm$^2$ | 1.7 µm$^2$ (4xX10x) |
| **T.S. of Leaf** | 3.2X2.2 µm | 2.0X1.8 µm (4xX10x) |
| **Oval shaped cystolith(Root)** | 1.2X0.8 µm | - |
| **Round cystoliths (stem)** | 0.8-1 µm (40xX10x) | 1.36X1.06 µm (40xX10x) |
| **Trichome (stem)** | Sessile glandular : 1.1 µm | Multicellular 4-5 µm (10xX10x) |
| **Round cystoliths (leaf)** | 1X0.8 µm (40xX10x) | 0.9 X0.9 µm (40xX10x) |
| **Candelabra trichomes (leaf)** | - | Stalk: 5 X 0.3µm (10xX10x) Head: 0.4 µm$^2$(10xX10x) |
| **Multicellular trichome (leaf)** | - | 2.9X0.3 µm (40xX10x) |
| **Sessile glandular trichome (leaf)** | 0.8 µm(40xX10x) | |

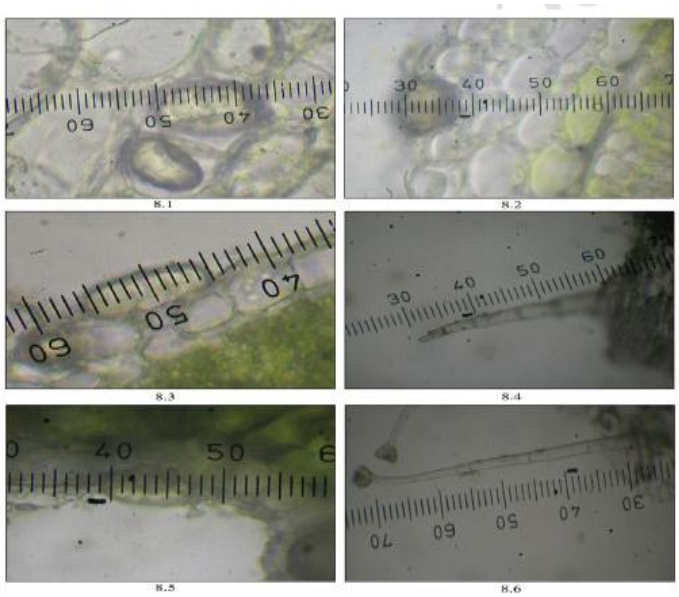

**Figure 8**

8.1 cystolith in *A.panniculata* root

8.2 round cystolith in *A. echioides* stem

8.3 sessile glandular head trichome *A.panniculata* stem

8.4 mulitcellur trichome *A. echioides* leaf

8.5 sessile glandular head trichome *A.panniculata* leaf

8.6 candelabra trichome *A. echioides* leaf

## DISCUSSION

From the morphological dissimilarity the plants can be easily distinguished like *A. echioides* (whole plant)was covered with minute hairs including the fruits, which was not so in case of *A. Panniculata.* Moreover the various micromorphological measurements further aids to the distinguishing factors.

Anatomically there was considerable change in the schematic diagram of both the plants when there transverse sections were observed. The T.S. of root showed presence of cysoliths in epiblema (wavy), cortex region in case of *A. pannniculata* which absent in case of *A.echioides,* moreover the xylem region occupies larger space in case of *A.echioides* then *A. pannniculata*.

Protuberances of the quadangular stem in T.S. is predominant and extended, trichomes were less and pith (pitted) occupies more space along with accumalation of microcrystals and oil golbules in case of *A. pannniculata* whereas in case of *A. echioides* proturbuurances are not that dominant, trichomes are more, xylem occupies more space and pith was devoid of eragastic substances.

On keen observation of T.S. of leaf passing through mid rib, ground tissue was present more in *A.echioides,* vascular bundle occupies more space in *A. pannniculata*. In case of *A. pannniculata* spongy parenchyma is compactly arranged without intracellullar spaces which was not so in case of *A.echioides.* In surface study, the presence of very few stomata in upper epidermis and presence of pleuricellular trichome in case of *A.echioides* which was not so in *A. pannniculata*.

Powder microsccopy of both shows similar characters; hence the wrong interpretation leads to adulteration of *A. pannniculata* with *A. echioides*. The only difference found in powder microscopic was group of simple starch grains are found in *A. echioides* which was absent in *A. pannniculata* and micro prismatic crystals which are present in *A. pannniculata* but devoid in case of *A. echioides.* Moreover the pollen grains present in *A. pannniculata* was dumbell shaped with two protuberances where as in *A. echioides* it was round with thick wall.

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